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Genetic variation in small proline rich protein 2B as a predictor for asthma among children with eczema

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ABSTRACT

Background: Small proline rich protein 2B (SPRR2B) is a skin and lung epithelial protein associated with allergic inflammation in mice that has not been evaluated in human atopic diseases.
Objective: To determine whether single-nucleotide polymorphisms (SNPs) in *SPRR2B* are associated with childhood eczema and with the phenotype of childhood eczema combined with asthma.
Methods: Genotyping for *SPRR2B* and filaggrin (*FLG*) was performed in 2 independent populations: the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS; N = 762; birth-age, 4 years) and the Greater Cincinnati Pediatric Clinical Repository (GCPCR; N = 1152; ages 5–10 years). Eczema and eczema plus asthma were clinical outcomes based on parental report and clinician's diagnosis. Genetic analyses were restricted to whites and adjusted for sex in both cohorts and adjusted for environmental covariates in CCAAPS.
Results: Variants in *SPRR2B* were not significantly associated with eczema in either cohort after Bonferroni adjustment. Children from both cohorts with the CC genotype of the *SPRR2B* rs6693927 SNP were at 4 times the risk for eczema plus asthma (adjusted odds ratio, 4.1; 95% confidence interval, 1.5–10.9; $P = .005$ in CCAAPS; and adjusted odds ratio, 4.0; 95% confidence interval, 1.8–9.1; $P < .001$ in the GCPCR), however. SNPs in *SPRR2B* were not in strong linkage disequilibrium with the R501X and del2282 *FLG* mutations, and these findings were independent of *FLG*.
Conclusions: An SNP in *SPRR2B* was predictive of asthma among white children with eczema from 2 independent populations. *SPRR2B* polymorphisms may serve as important predictive markers for the combined eczema plus asthma phenotype.

Eczema is a chronic inflammatory skin condition commonly associated with other atopic diseases, such as asthma.[1,2] Genetic variants in epidermal proteins, such as filaggrin (FLG), are thought to play a critical role in the development of eczema and have been associated with the combination of eczema plus asthma.[2–6] The *FLG* gene is located within the epidermal differentiation complex, a region on chromosome 1q21 that also encodes for numerous other proteins involved in maintaining the skin barrier.[7–10]

Linkage and expression studies indicate that *FLG* alone does not account for the strong association between region 1q21 and eczema nor for the possible association between this region and the combined phenotype of eczema plus asthma.[4,11,12] Small proline rich proteins (SPRRs) are cross-linking components of the keratinocyte-cornified envelope encoded on 1q21 that may be critically important to the formation of the skin barrier.[13,14] SPRR2B in particular has been shown to be induced by allergens and the T_H2 cytokine, interleukin 13, in mouse models of asthma and allergic gastrointestinal disease.[15,16] Moreover, SPRR2B messenger RNA

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expression is increased during asthma exacerbations, and large allele frequency differences exist between human populations with high rates of asthma vs those with a lower prevalence of the disease.[17,18] Together, these findings suggest that SPRR2B may play a role in both eczema and asthma.

We hypothesized that polymorphisms in *SPRR2B* would be significantly associated with childhood eczema and with the eczema plus asthma phenotype. To test this hypothesis, we examined differences in the frequency of single-nucleotide polymorphisms (SNPs) in *SPRR2B* between children with and without eczema, and eczema plus asthma who were participating in the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS) and the Greater Cincinnati Pediatric Clinic Repository (GCPCR) at Cincinnati Children's Hospital Medical Center.[18,19]

Methods

CCAAPS is a longitudinal birth cohort at high risk for atopy.[19] Newborns in the Cincinnati metropolitan area were identified by public birth records from 2001 to 2003. All 762 infants had a parent with symptoms of asthma, allergic rhinitis, or eczema and with at least one positive skin prick test result to a panel of 15 aeroallergens. Parents signed an informed consent approved by the institutional review board of the University of Cincinnati. The GCPCR (N = 1152) includes children 5 to 17 years of age from subspecialty clinics at Cincinnati Children's Hospital and control subjects from the general population.[18] Parents signed an informed consent approved by the Cincinnati Children's Hospital institutional review board. (Further details are provided in Table 1 and in the eMethods.)

Outcome measures

Eczema. The definition of eczema in CCAAPS was adapted from a validated questionnaire (International Study of Asthma and Allergies in Childhood) and included a parental report of the child's scratching *and* redness, "raised bumps," or dry skin/scaling for *at least 6 of the last 12 months*.[20] Children who tested positive for eczema at any point from 1 through 4 years of age were considered cases. This definition was established a priori and was previously used in CCAAPS.[21] For the GCPCR, cases with eczema included children with a physician's diagnosis or a parental report of eczema. A separate analysis with eczema based on the clinician's diagnosis at 1, 2, 3, or 4 years of age was performed in CCAAPS. Physical examination findings considered to be consistent with eczema included erythema, papulation, excoriations, and/or lichenification.

Eczema with asthma. Having eczema at any point from 1 through 4 years of age combined with asthma at the age of 4 years served as a secondary outcome measure for CCAAPS.[2] Given that objective testing for asthma could not be performed in CCAAPS due to the age of the children, a case definition for asthma at the age of 4 years was developed; this case definition included a parental report of persistent wheezing (≥2 wheezing episodes not associated with a cold or upper respiratory tract infection) at both 3 *and* 4 years of age or a diagnosis of asthma by a child's physician by 4 years of age.[22] Although this case definition is not asthma per se, for the sake of simplicity it will be referred to as *asthma* for the remainder of the article. The reference group for the eczema plus asthma outcome included children with neither condition or just eczema or asthma alone (but not both). For the GCPCR, cases included children with both eczema and asthma, whereas the reference group had neither condition or just eczema or asthma alone. In both CCAAPS and the GCPCR, a separate analysis was performed in which only children with neither eczema nor asthma were included in the reference group. (Additional details are provided in the eMethods.)

Selection of SNPs

A priori, we decided to use the GCPCR as a replication cohort. Therefore, SNPs evaluated in CCAAPS were the same as those analyzed concurrently in the GCPCR. The *SPRR2B* SNPs chosen included 1 intronic tagging SNP (rs6693927), 2 SNPs in the 5′ and 3′ untranslated regions (rs7525198 and rs6673356, respectively), and a missense mutation (rs404408).[18] Tagging SNPs and SNPs in regulatory regions with minor allele frequency greater than 5% across the *SPRR2B* gene were chosen to adequately represent the gene, as previously described.[23]

Genotyping

SPRR2B genotyping for CCAAPS and the GCPCR was accomplished using a LightTyper platform and a custom Illumina Golden Assay, respectively.[18,24,25] The rs6693927 and rs7525198 SNPs were genotyped with error rates of less than 2% and were in Hardy Weinberg equilibrium (eTable 1). The rs6673356 and rs404408 SNPs were not included in further analyses because genotyping results did not meet quality controls. Genotyping of *FLG* R501X was accomplished by polymerase chain reaction and restriction fragment length polymorphism in CCAAPS and the GCPCR as previously described (eMethods).[6,26] Primer sequences and genotyping methods for *FLG* 2282del4 in the GCPCR, as well as further information regarding genotyping of *SPRR2B*, are provided in the online supplement.

Table 1
Description of the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) and the Greater Cincinnati Pediatric Clinic Repository (GCPCR)

Variable	CCAAPS	GCPCR
Type of cohort	Longitudinal birth cohort study of children from atopic parents	Children from subspecialty clinics at an academic, pediatric medical center and control children from the general population
Total No. of children	762	1,152
Age of children at time of data collection, y	1–4	5–17 (5- to 10-year-olds analyzed)
Study population	Identified from birth records (2001–2003) in Cincinnati and Northern Kentucky and (a) had at least 1 parent with symptoms of asthma, allergic rhinitis, or eczema and positive skin prick test results to 1/15 aeroallergens and (b) lived <400 m or > 1,500 m from a major road	Cases recruited from allergy/immunology, pulmonary, and dermatology clinics. Some controls recruited from headache, plastics, and orthopedics clinics; some controls recruited from the general population in Cincinnati.
Definition of eczema	Primary: a parental report of the child's scratching and redness, "raised bumps," or dry skin/scaling for at least 6 of the last 12 months; Secondary: clinician's diagnosis	A physician's diagnosis or a parental report of eczema
Definition of asthma	A parental report of persistent wheezing (≥2 episodes not associated with a cold or upper respiratory tract infection) at both 3 and 4 years of age or a physician's diagnosis of asthma by 4 years of age	A physician's diagnosis of asthma

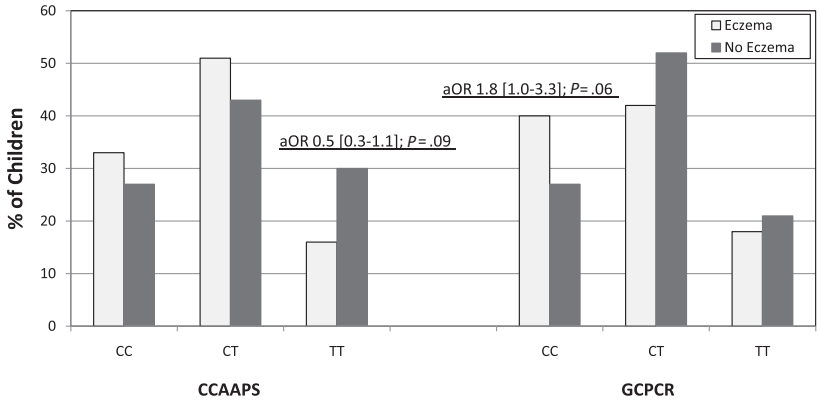

Fig. 1. Genotypes of rs6693927 and eczema among whites in the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS) (1–4 years of age) and Greater Cincinnati Pediatric Clinical Repository (GCPCR) (5–10 years of age) cohorts. The figure shows the percentage of children with genotypes of the rs6693927 SNP based on whether they had eczema. Adjusted odds ratios (aORs) for statistically significant or borderline effects are given. Data from CCAAPS were adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin (eTable 3). Data from the GCPCR were adjusted for sex.

Statistical analysis

SAS statistical software (SAS Institute Inc, Cary, North Carolina) was used to perform multivariate logistic regression to adjust findings in CCAAPS for host and environmental covariates, including income under $50,000, breastfeeding (yes/no), dog and/or cat ownership (yes/no based on home visit), and house dust endotoxin exposure at 1 year of age (based on home visit). All potential covariates listed in eTable 2 were initially included in the maximum likelihood estimated model and removed if $P > .15$. Analyses in the GCPCR were adjusted for sex. Environmental covariates from the GCPCR were not available at the time of this analysis. Analyses in both cohorts were restricted to whites given the small sample size of African Americans. A priori, children older than 10 years were excluded from the analysis in the GCPCR to improve comparability with CCAAPS and to decrease recall bias related to transient eczema occurring at younger ages.[27]

A genotypic analysis for *SPRR2B* was performed. This model was chosen given that the mode of inheritance is not known and an additive model may be less likely to detect an association with relevant outcomes in the case of recessive traits.[28] To perform a genotypic analysis, we used logistic regression to calculate adjusted odds ratios (aORs), 95% confidence intervals (CIs), and *P* values that represented the relationship between genotypes of rs6693927 and rs7525198 (eg, TT vs all others; CC vs all others; CT vs all others) and the outcomes of eczema and eczema plus asthma. For rs6693927, TT represents a homozygous recessive with 2 TT alleles, CT represents a heterozygote, and CC represents a homozygous recessive with 2 CC alleles. A haplotype analysis using PLINK software was also conducted to determine whether there were additional effects from combined genotypes of rs6693927 and rs7525198.[29] Linkage disequilibrium (LD) (D′ and r^2) between *SPRR2B* and *FLG* variants was estimated using Haploview.[30] A LD-adjusted Bonferroni calculation (0.03) was applied to genetic findings to account for multiple comparisons.

Results

Study population characteristics

In the CCAAPS cohort, 161 of 396 whites (41%) had eczema for at least 1 year from 1 through 4 years of age, whereas 59 of 328 whites (18%) ages 5 to 10 years in the GCPCR had the disease (Table 2). Among whites with eczema from CCAAPS, 144 of 161 (89%) were atopic based on 1 positive skin prick test result to a panel of 15 aeroallergens at any point from 1 through 4 years of age; skin prick test results were not available for the GCPCR. Findings related to host and environmental covariates in CCAAPS are presented in eTable 3.

Genetic predictors of eczema

Among whites from CCAAPS, the TT genotype of the rs6693927 SNP (TT_rs6693927) in *SPRR2B* was protective against eczema after adjustment for sex (aOR, 0.5; 95% CI, 0.2–0.9; $P = .03$); however, this finding did not meet criteria for significance based on LD-adjusted Bonferroni correction ($P < .03$ was significant). There was a trend toward an association with eczema after adjustment for environmental covariates (aOR, 0.5; 95% CI, 0.3–1.1; $P = .09$; Fig 1). Findings for TT_rs6693927 in the GCPCR study population were not statistically significant (Fig 1 and eTable 4). The *SPRR2B* rs7525198 SNP was not associated with eczema in either CCAAPS or the GCPCR. In addition, the combination of rs7525198 and rs6693927 was not more predictive of eczema than rs6693927 alone, based on findings from combined genotype (diplotype) and haplotype analyses (data not shown).[29]

Genetic predictors of the eczema plus asthma phenotype

We next evaluated the association between polymorphisms in *SPRR2B* and the presence of both eczema and asthma.[2] As indicated in Table 2, 25 whites (7%) from CCAAPS and 28 whites (9%) from the GCPCR had both conditions. An odds ratio for TT_rs6693927 could not be calculated for CCAAPS because none of the children with this genotype had the phenotype of eczema plus asthma; the odds ratio for the CC genotype of the rs6693927 SNP (CC_rs6693927) was therefore calculated instead. Among whites from both CCAAPS and

Table 2
White children meeting criteria for eczema with and without asthma in the Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) (1–4 years of age) and Greater Cincinnati Pediatric Clinic Repository (GCPCR) (5–10 years of age) Cohorts

Characteristic	No. (%) of children	
	CCAAPS	GCPCR
Any eczema	161 (41)[a]	59 (18)[b]
No eczema	235	269
Eczema plus asthma	25 (7)	28 (9)
No eczema or asthma	195 (56)	111 (34)
Eczema but no asthma	114 (33)	31 (10)
No eczema with asthma	16 (5)	158 (48)

[a]Sufficient information to determine whether white children met criteria for eczema (1–4 years of age) was available for 396 children from CCAAPS; information for both eczema (1–4 years of age) and asthma (4 years of age) was available for 350 children. Because of this, the denominator used to determine the percentage of children with eczema is 396, whereas the denominator used for other percentages for CCAAPS is 350.

[b]Sufficient information to determine whether white children met criteria for eczema and/or asthma was available for 328 children from the GCPCR. The denominator was 328 for all percentage calculations for the GCPCR.

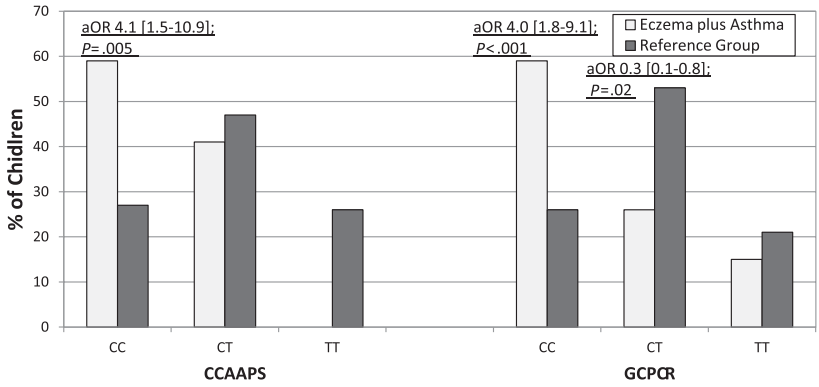

Fig. 2. Genotypes of rs6693927 and the eczema plus asthma phenotype among whites in the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS) (1–4 years of age) and Greater Cincinnati Pediatric Clinical Repository (GCPCR) (5–10 years of age) cohorts. The figure shows the percentage of children with genotypes of the rs6693927 single-nucleotide polymorphism based on whether they had the combination of eczema and asthma. The reference group for this analysis included children with neither condition or just with eczema or asthma (but not both). Adjusted odds ratios (aORs) are displayed for statistically significant effects. Data from CCAAPS were adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin (eTable 3). Data from the GCPCR were adjusted for sex.

the GCPCR, CC_rs6693927 conferred a 4-fold increased risk for the eczema plus asthma phenotype (aOR, 4.1; 95% CI, 1.5–10.9; $P = .005$ after adjustment for host and environmental covariates from the logistic regression model in CCAAPS; and aOR, 4.0; 95% CI, 1.8–9.1; $P < .001$ after adjustment for sex in the GCPCR; Fig 2 and eTable 5). These findings met the LD-adjusted Bonferroni criteria for significance ($P < .03$). As was the case with eczema, rs7525198 did not confer significant effects for eczema plus asthma by itself, and the combination of rs7525198 and rs6693927 was not more predictive of eczema plus asthma than the latter SNP alone (data not shown).[29]

Additional analyses based on different definitions of eczema and eczema plus asthma

As a secondary analysis, we also evaluated eczema defined solely by clinician's diagnosis at 1, 2, 3, or 4 years of age in CCAAPS. Findings were similar to those seen with the survey definition of eczema in CCAAPS. Among whites ages 1 to 4 years, 117 (44%) had clinician-diagnosed eczema and 149 (56%) did not. There was a possible protective effect from clinician-diagnosed eczema conferred by the TT_6693927 genotype after adjustment for host and environmental covariates (aOR, 0.6; 95% CI, 0.4–1.1; $P = .10$; Fig 3); however, this finding was not statistically significant.

We also evaluated the relationship between the eczema plus asthma phenotype using the clinician's diagnosis of eczema definition in CCAAPS. Findings were strikingly similar to those seen with the survey definition of eczema. There were 16 children (7%) with the clinician's diagnosis of eczema plus asthma and 217 children (93%) in the reference group. A strong association was found between the CC_6693927 genotype and the combination of clinician-diagnosed eczema plus asthma (aOR, 5.3; 95% CI, 1.6–17.4; $P = .005$ after adjustment for host and environmental covariates from the logistic regression model; Fig 4). These findings met the LD-adjusted Bonferroni criteria for significance ($P < .03$).

To further clarify the relationship between polymorphisms in SPRR2B and the eczema plus asthma phenotype, an additional analysis was conducted in which the reference group included only children with neither eczema nor asthma (n = 195 in CCAAPS and n = 100 in the GCPCR). Findings from this analysis were nearly identical to those previously reported. That is, CC_rs6693927 conferred increased risk for eczema plus asthma (aOR, 4.5; 95% CI, 1.6–13.1; $P = .005$ after adjustment for host and environmental covariates from the logistic regression model in CCAAPS; and aOR, 4.6; 95% CI, 1.9–11.3; $P < .001$ in the GCPCR). These findings met the LD-adjusted Bonferroni criteria for significance ($P < .03$).

Association between rs6693927 and asthma

To better understand the relationship between the rs6693927 SNP and the eczema plus asthma phenotype, we also evaluated the

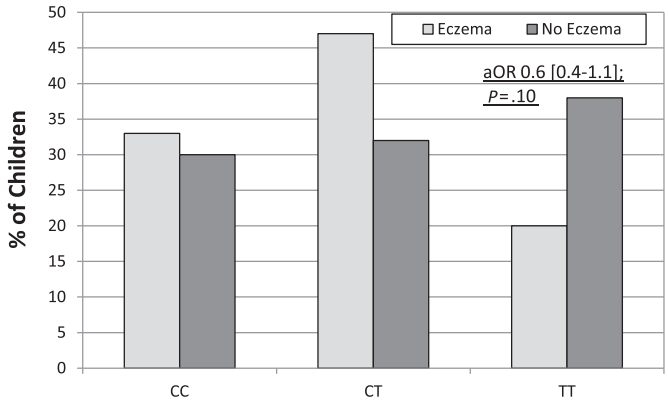

Fig. 3. Genotypes of rs6693927 and clinician-diagnosed eczema among whites in the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS) cohort (1–4 years of age). The figure shows the percentage of whites from CCAAPS with genotypes of the rs6693927 single-nucleotide polymorphism based on whether they had clinician-diagnosed eczema. Data were adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin.

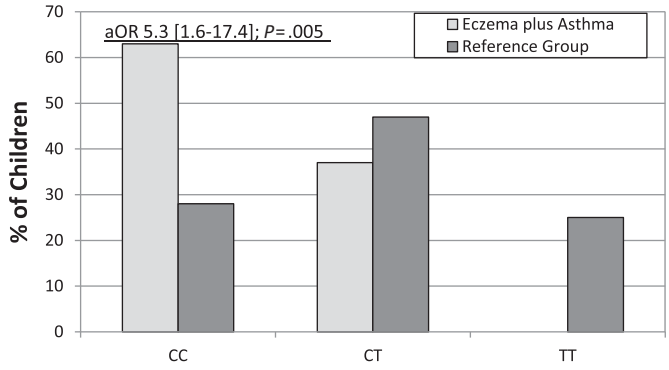

Fig. 4. Genotypes of rs6693928 and the clinician-diagnosed eczema plus asthma phenotype among whites from Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS) (1–4 years of age). The figure shows the percentage of whites from CCAAPS (ages 1–4) with genotypes of the rs6693927 single-nucleotide based on whether they had the combination of clinician-diagnosed eczema and asthma. The reference group for this analysis included children with neither condition or just with eczema or asthma (but not both). Odds ratios adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin are displayed for statistically significant effects.

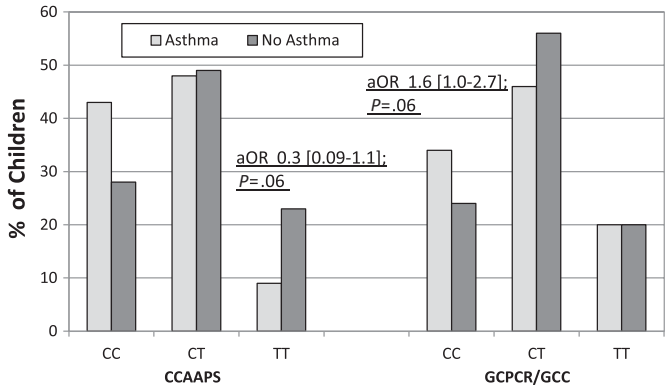

Fig. 5. Genotypes of rs6693928 and asthma among whites in the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS) (1–4 years of age) and the Greater Cincinnati Pediatric Clinical Repository (GCPCR) (5–10 years of age). The figure shows the percentage of whites from CCAAPS and the GCPCR with genotypes of the rs6693927 SNP based on whether they had asthma. Data from CCAAPS were adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin. Data from the GCPCR were adjusted for sex.

relationship between this SNP and asthma alone (without eczema). Among whites from CCAAPS, there was a trend toward a protective effect from asthma conferred by the TT_6693927 genotype after adjustment for host and environmental covariates (aOR, 0.3; 95% CI, 0.09–1.1; $P = .06$; Fig 5). Among whites 5 to 10 years of age from the GCPCR, there was also a trend toward increased risk for asthma alone conferred by the CC_6693927 genotype (aOR, 1.6; 95% CI, 1.0–2.7; $P = .06$).

Determination of the relationship to FLG polymorphisms

Given the previously reported association between *FLG* null mutations and the eczema plus asthma phenotype and that both *FLG* and *SPRR2B* are located on chromosome 1q21, we next determined whether the effects of SPRR2B were related to LD with *FLG*. *FLG* null mutations were not in strong LD with *SPRR2B* polymorphisms ($r^2 < .05$, $D' < .68$ for R501X and 2282del4 in the GCPCR, with similar findings for R501X in CCAAPS).

Discussion

Evidence from the literature has established that epidermal barrier function plays a critical role in eczema and possibly in the phenotype of eczema plus asthma.[1,2,5] The combination of eczema plus asthma may represent a distinct endophenotype with unique pathogenic and prognostic implications.[5,31] Identification of risk factors that may modify the risk of asthma among children with eczema is important.[31,32] In addition, improved understanding of the relationship between eczema and asthma may provide mechanistic insights into the pathogenesis of asthma.[5,32] FLG mutations alone are unlikely to account for the strong genetic linkage between the epidermal differentiation complex on chromosome 1q21 and the eczema plus asthma phenotype.[1,2] Given this premise and based on preliminary data from RNA expression studies and allele frequency differences in asthmatic populations, we explored the relationship among childhood eczema, the combination of eczema plus asthma, and SPRR2B, an epithelial protein encoded on 1q21.[17,18] We demonstrated a possible association between an SNP in *SPRR2B* and eczema among whites 4 years and younger from the CCAAPS cohort; however, this finding was not statistically significant after Bonferroni correction. A nonsignificant trend toward an association between this SNP and asthma was also seen in both CCAAPS and the GCPCR. Interestingly, in both cohorts, the CC genotype of the rs6693927 SNP conferred strong, statistically significant effects among children who had the eczema plus asthma phenotype. That is, the rs6693927 CC genotype conferred an approximately 4-fold increased risk for eczema plus asthma in both CCAAPS and the GCPCR. Findings for this SNP did not appear to be related to *FLG* polymorphisms. Thus, polymorphisms in *SPRR2B* may independently serve as useful markers to predict the development of asthma among children with eczema.

Mechanistic studies to explain the reasons for an association between *SPRR2B* polymorphisms and the eczema plus asthma phenotype have yet to be conducted. Alterations in SPRR2B may potentially result in a defective skin barrier. Small proline rich proteins, including SPRR2B, play an important role in the formation of the keratinocyte-cornified envelope.[33] After cross-linking by transglutaminases, they serve as the scaffold on which other epithelial proteins are added.[34] It is not clear, however, whether the link between SPRR2B and asthma is entirely mediated by effects on the skin barrier. In contrast to FLG, which is not expressed in the lungs, SPRRs are expressed in multiple tissues throughout the body. SPRR2B in particular is expressed in the lungs of mice, and its expression is specifically altered in the nasal epithelium of asthmatic patients.[15,17,34] It has also been speculated that the altered expression of SPRR genes as part of a stress response to UV light, infection, or allergic inflammatory signals may reflect a role in tissue repair.[34–36] This implies that quantitative or functional abnormalities involving SPRRs might lead to a dysfunctional repair response with the potential for pathologically adverse outcomes. Alternatively, up-regulation of SPRR2B during asthma exacerbations may reflect another undiscovered role of this protein in allergic inflammation.[17,34]

Although there is a possible association between the rs6693927 polymorphism in *SPRR2B* and eczema and a strong relationship with the combination of eczema and asthma, there are limitations to what may be concluded from these findings. It is possible that this association reflects genetic linkage with other epidermal barrier proteins encoded on region 1q21, besides FLG, and/or that SPRR2B is coexpressed with other SPRRs that are responsible for clinically meaningful responses. The rs6693927 SNP has not been clearly related to changes in SPRR2B protein function or expression, and the possibility exists that this SNP may be a *marker* of disease causing mutations rather than disease causing itself. Another possible limitation is that there were relatively small numbers of children with the eczema plus asthma phenotype from both cohorts. There were also differences in the cohorts studied, including that they enrolled children of different ages and that CCAAPS is a high-risk birth cohort, whereas the GCPCR includes both atopic and control children. Differences in the prevalence of eczema between the cohorts are reflective of these factors. Findings related to asthma in the CCAAPS cohort will be validated once the children are old enough to undergo objective testing. Despite these differences, the prevalence of the eczema plus asthma phenotype was similar between cohorts; further, odds ratios for the effects of *SPRR2B* polymorphisms were almost identical. In addition, findings were similar despite different outcome definitions between cohorts and were comparable in CCAAPS regardless of the outcome definition used.

In conclusion, the rs6693927 SNP in *SPRR2B* was significantly associated with the eczema plus asthma phenotype among whites from both cohorts. It was not significantly associated with eczema in CCAAPs or in the GCPCR. Findings were independent of *FLG* polymorphisms and were robust despite potential differences in the cohorts.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.anai.2012.01.004.

eMethods

Study participant recruitment and study design for the Cincinnati Childhood Allergy & Air Pollution Study (CCAAPS)

CCAAPS is a longitudinal birth cohort study investigating possible associations between air pollution and allergy. Newborns in the Cincinnati metropolitan area were identified by public birth records from 2001 to 2003.[1,2] All 762 infants were required to have a parent with symptoms of asthma, allergic rhinitis, or eczema and with at least 1 positive skin prick test (SPT) result to a panel of 15 aeroallergens.

Parents of children participating in CCAAPS completed yearly in-person surveys at 1 through 4 years of age. Physical examinations and a clinician's assessment were performed on the same day as the in-person surveys. Children were skin tested by trained clinicians for 15 aeroallergens on a yearly basis from ages of 1 through 4 years.[1] Buccal swabs (Zymo Research Genomic DNA II Kit or Purgene DNA Purification System; Gentra System, Minneapolis, Minnesota) or saliva samples (Oragene DNA Self-Collection Kit; DNA Genotek Inc, Kanata, Ontario, Canada) were collected for DNA. A home visit and environmental assessment were conducted before 1 year of age. House dust samples were collected from a 2-m^2 area of floor surface from the infant's primary living area using a Filter Queen Majestic vacuum cleaner at a rate of 2 min/m^2. Endotoxin and (1→ 3) β-D-glucan concentrations in house dust were determined using a Limulus amebocyte lysate assay.[3,4] Pyrochrome modification of the assay was used for endotoxin, and Glucatell modification was used for (1→ 3) β-D-glucan.[3,4]

Study participant recruitment and study design for the Greater Cincinnati Pediatric Clinical Repository (GCPCR)

Children from the GCPCR were recruited from allergy/immunology, pulmonary, dermatology, headache, plastics, and orthopedics clinics at Cincinnati Children's Hospital Medical Center (CCHMC), as well as from the community at large, using paper and online advertising media. In addition, to include a sufficient number of children who were unaffected by eczema, some control children were drawn from the Genomic Control Cohort (GCC) at CCHMC. The GCC is a representative sample of more than 1,000 children from the general population, recruited using community-based strategies from 7 counties comprising the Greater Cincinnati region. Data for children 5 to 17 years of age were available at the time of this analysis. Children older than 10 years, however, were excluded from the analysis to improve comparability with the CCAAPS cohort and to decrease recall bias related to transient eczema occurring at younger years.[5]

After providing informed consent, parents were asked to complete clinical questionnaires at their initial clinic visit and all subsequent visits. Children provided a buccal swab or saliva sample for DNA (collection kits were the same as those listed above for CCAAPS).[6]

Supplemental information for outcome measures

The definition of eczema in the CCAAPS cohort was adapted from a validated questionnaire (International Study of Asthma and Allergies in Childhood) and included a parental report of the child's scratching *and* redness, "raised bumps," or dry skin/scaling for at least 6 of the last 12 months.[7] This definition of eczema was established a priori and was previously used in CCAAPS.[8] Children who tested positive for eczema at any point from 1 through 4 years of age were considered cases. To be included in the reference group, children had to test negative for eczema based on both the questionnaire and the clinician's assessment. To avoid potential misclassification, children who did not have eczema based on parental report but did have eczema based on the clinician's assessment were therefore excluded from the analysis.[8] Physical examination findings considered to be consistent with eczema included erythema, papulation, excoriations, and/or lichenification. Given that the prevalence of eczema is highest before 3 years of age, children with missing eczema data at 1 or 2 years of age (and who were not diagnosed as having eczema at 3 or 4 years of age) were excluded from the analysis.[5]

For the GCPCR, cases with eczema included children with either a physician's diagnosis or a parental report of eczema. The reference group for the GCPCR included children with no personal or family history of eczema (regardless of other atopic conditions) based on parental report.

Genotyping

SPRR2B genotyping for CCAAPS was accomplished using a LightTyper platform (Roche Diagnostics, GmbH, Mannheim, Germany) to perform fluorescent melting curve analysis.[9,10] In brief, fluorescently labeled sequence-specific oligonucleotide probes were added to DNA extracted from buccal swabs or saliva before polymerase chain reaction (PCR). After DNA amplification by PCR, the amplicon/probe heteroduplex was slowly heated, and changes in fluorescence were measured to generate a melting curve. Genotypes were determined based on characteristic melting curves. The rs6673356 and rs404408 single-nucleotide polymorphisms (SNPs) were not included in further analyses because genotyping results did not meet quality controls. Adequate separation of peaks to distinguish genotypes could not be achieved with the LightTyper for rs6673356 in CCAAPS. The rs404408 SNP (representing a null allele) could not be accurately genotyped using either the LightTyper (CCAAPS) or Illumina assay (GCPCR).

Genotyping of *FLG* R501X was accomplished by PCR and restriction fragment length polymorphism in both CCAAPS and the GCPCR as previously described.[11,12] Briefly, PCR was performed with the primers 5′-CTG GAG GAA GAC AAG GAT CG-3′ and 5′-TTG TCT GCT TGC ACT TCT GG-3′. The PCR product was then digested with restriction endonuclease Nla III followed by electrophoresis on a 4% agarose gel. An unmutated allele digested into fragments of 213 and 32 bp, whereas a mutated allele digested into fragments of 176, 37, and 32 bp. *FLG* 2282del4 genotyping in the GCPCR/GCC included PCR with primers 5′-TCCCGCCACCAGCTCCA-3′, 5′-**TGTAAAACGACGGCCAGT**CTGATGGTGACCAGCCTGT3', and *__TGTAAAACGACGGCCAGT__ (bold indicates M13-21 sequencing primer and asterisk indicates fluorophore) followed by analysis using the Fragment Sizing Protocol (AB Biosystems 3730xL DNA Analyzer; Applied Biosystems, Carlsbad, California). Each set of 96 PCR reactions included controls for each genotype and a negative control, all of which performed as expected. *FLG* 2282del4 was not genotyped in CCAAPS.

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eTable 1
SPRR2B genotyping for CCAAPS and the GCPCR/GCC

	No. of children					
	rs6693927			rs7525198		
	CC	CT	TT	CC	CA	AA
CCAAPS						
White	97	155	71	274	42	1
African American	54	24	6	45	29	7
Total	151	179	77	319	71	8
GCPCR						
White	177	274	113	507	62	2
African American	180	100	16	141	121	31
Race unknown/other	15	13	2	16	13	1
Total	372	387	131	664	196	34

Abbreviations: CCAAPS, Cincinnati Childhood Allergy & Air Pollution Study; GCPCR, Greater Cincinnati Pediatric Clinical Repository; *SPRR2B*, small proline rich protein 2B.

eTable 2
Potential covariates in estimated maximum model

Potential covariate	Age of child when data collected	Characteristics of variable
Sex	Enrollment	
Race	Enrollment	African American vs all others
Parental income	Enrollment	9 categories, lowest = <$9,999, highest = >$110,000; ordinal
Highest level of parental education attained	Enrollment	Categorical; analyzed separately by sex
History of parental eczema	Enrollment	Yes/no
History of parental asthma	Enrollment	Yes/no
Dog ownership	Before 1 year of age (home visit)	Yes/no
	Dog allergen (Can f 1) levels: home visit	Continuous and in tertiles
Cat ownership	Before 1 year of age (home visit)	Yes/no
	Cat allergen (Fel d 1) levels: home visit	Continuous and in tertiles
Endotoxin	Before 1 year of age (home visit)	Continuous and in tertiles
βa-Glucan	Before 1 year of age (home visit)	Continuous and in tertiles
Dust mite (Der p 1)	Before 1 year of age (home visit)	Continuous and in tertiles
Season of birth	Enrollment	Winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug), fall (Sep-Nov), Ordinal
Breastfeeding	2 years of age (questionnaire)	Yes/no and months breastfed
Tobacco smoke exposure	1–4 years of age (questionnaire)	3 variables: (1) total No. of smokers, (2) cigarettes smoked in household, and (3) cigarettes smoked by mother; analyzed as none vs any and No. of cigarettes smoked
Elemental carbon attributable to traffic	Average during first year of life	Measures exposure to traffic related particles; continuous and in tertiles
Daycare attendance	1–4 years of age (questionnaire)	Yes/No (each year analyzed separately)
No. of siblings	1–2 years of age (questionnaire)	Analyzed as an ordinal and a continuous variable
Timing of food introduction	6 months of age, 1–4 years of age (questionnaire)	Egg and milk: before vs after 1, 2, 3, or 4 years of age, respectively Nut (peanut or tree nut): before vs after 2 or 3 years of age, respectively

eTable 3
Characteristics of white patients 1 to 4 years of age from CCAAPS included in the analysis (N = 396)

Characteristic	No. with eczema/total No. with characteristic (% with eczema)	Unadjusted OR (95% CI) for eczema; *P* value	Adjusted OR (95% CI) for eczema; *P* value
Sex		1.1 (0.7–1.6); .80	0.9 (0.5–1.6);.60
Male	91/221 (41)		
Female	70/175 (40)		
Income <$50,000 annually	56/130 (43)	1.2 (0.8–1.8); .50	0.9 (0.5–1.6); .60
Breastfeeding	119/286 (42)	1.3 (0.8–2.1); .30	1.4 (0.7–2.9); .30
Pet keeping			
Dog	53/157 (34)	0.6 (0.4–1.0]; .03	0.6 (0.4–1.0); .03
Cat	36/105 (34)	0.7 (0.4–1.1); .10	0.7 (0.5–1.2); .30
Endotoxin exposure			
Highest quartile	40/99 (40)	1.0 (0.56–1.6); >.99	1.0 (1.0–1.0); .80[a]
Lowest quartile	38/106 (36)	0.8 (0.5–1.2); .20	

Abbreviations: CCAAPS, Cincinnati Childhood Allergy & Air Pollution Study; CI, confidence interval; OR, odds ratio.
[a]Endotoxin was analyzed continuously in the multivariable analysis.

eTable 4
Genotype frequencies for rs6693927 and eczema among white patients in CCAAPS and the GCPCR

	CC	CT	TT
CCAAPS Patients (1–4 Years Old)			
Total with genotype	65	102	54
Eczema	29	44	14
No eczema	36	58	40
OR (95% CI) for eczema adjusted for sex; *P* value	1.3 (0.7–2.4); .30	1.3 (0.8–2.3); .30	0.5 (0.2–0.9); .03
OR (95% CI) for eczema adjusted for environmental covariates[a]; *P* value	1.4 (0.8–2.7); .30	1.2 (0.7–2.1); .60	0.5 (0.3–1.1); .09
GCPCR Patients (5–10 Years Old)			
Total with genotype	88	150	61
Eczema	22	23	10
No eczema	66	127	51
OR (95% CI) for eczema adjusted for sex; *P* value	1.8 (1.0–3.3]; .06	0.7 (0.4–1.2); .20	0.8 (0.4–1.8); .60

Abbreviations: CCAAPS, Cincinnati Childhood Allergy & Air Pollution Study; CI, confidence interval; GCPCR, Greater Cincinnati Pediatric Clinical Repository; OR, odds ratio.
[a]Adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin.

eTable 5
Genotype frequencies for rs6693927 and the combination of eczema and asthma among patients in CCAAPS and the GCPCR

	CC	CT	TT
CCAAPS Patients (1–4 Years Old)			
Total with genotype	61	95	49
Eczema plus asthma	10	7	0
Reference group[a]	51	88	49
OR (95% CI) for eczema plus asthma adjusted for sex; *P* value	3.9 (1.4–10.7); .009	0.8 (0.3–2.2); .70	Cannot calculate
OR (95% CI) for eczema plus asthma adjusted for environmental covariates[b]; *P* value	4.1 (1.5–10.9); .005	0.9 (0.3–2.6); .90	Cannot calculate
GCPCR Patients (5–10 Years Old)			
Total with genotype	88	150	61
Eczema plus asthma	16	7	4
Reference group[a]	72	143	57
OR (95% CI) for eczema plus asthma adjusted for sex; *P* value	4.0 (1.8–9.1); <.001	0.3 (0.1–0.8); .02	0.6 (0.2–2.0); .40

Abbreviations: CCAAPS, Cincinnati Childhood Allergy & Air Pollution Study; CI, confidence interval; GCPCR, Greater Cincinnati Pediatric Clinical Repository; OR, odds ratio.
[a]The reference group for this analysis included children with either eczema or asthma or neither condition.
[b]Adjusted for sex, income, breastfeeding, cat ownership, dog ownership, and endotoxin.